Oral Presentation Abstracts

Connecting Research and Practice: A Dialogue between ATSDR and the NIEHS Superfund Research Program

Session One: Legacy Contaminants

Arsenic Exposure and Mortality in Bangladesh: Findings from the Health Effects of Arsenic Longitudinal Study

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Background: Chronic arsenic exposure through drinking water has become a growing public health issue affecting millions of people worldwide, including 35 to 77 million in Bangladesh and nearly 15 million in the United States. Inorganic arsenic is a class I human carcinogen and puts exposed individuals at increased risk of skin and other arsenic-induced cancers, as well as cardiovascular, pulmonary, and other non-malignant disorders. As part of the Columbia University Superfund Research Program, we established the Health Effects of Arsenic Longitudinal Study (HEALS), a large prospective cohort study based on individual-level data among a population exposed to a wide range of inorganic arsenic from drinking water in Araihazar, Bangladesh. Over the past ten years, using a population-based sampling frame, we recruited over 20,000 men and women (with > 97% response rates) and collected detailed questionnaires, clinical data, and biospecimen samples from them at baseline and biennially thereafter. Through a dedicated medical clinic, established for the project, we have also developed an effective mechanism of following the cohort, especially for detecting incidence and mortality of dermatological, pulmonary, and cardiovascular disorders.

Objectives: Utilizing data from the HEALS cohort, we evaluated the association between chronic arsenic exposure and all-cause, chronic disease, cardiovascular disease, and lung disease mortalities using a prospective design and individual-level assessment of arsenic exposure.

Design: Study participants, aged 18-75 years at enrollment, have been chronically exposed to arsenic at various doses through the consumption of groundwater. Individual-level arsenic exposure was measured at baseline by well water arsenic and urinary total arsenic concentrations. Vital status was assessed and cause of death was determined using a verbal autopsy interview. Cox proportional hazard regression models were used to estimate hazard ratios (HRs) and their 95% confidence intervals (CI) for various categorizations of mortality with respect to arsenic exposure.

Results: We observed that those exposed to the highest concentration of arsenic in water (150.1-864.0 µg/L) were at nearly 70% higher risk for all-cause mortality compared to those exposed to less than 10.0 µg/L. There was also a dose-response relation between arsenic in water and mortality from ischaemic heart disease with HRs in increasing quartiles of arsenic concentration of 1.00 (reference), 1.22 (0.65 to 2.32), 1.35 (0.71 to 2.57), and 1.92 (1.07 to 3.43) (P = 0.0019 for trend), respectively, after adjustment for potential confounders. Additionally, a 1 standard deviation increase in urinary total arsenic concentration

was associated with a 16% increase in lung disease mortality (95% CI: 1.06, 1.28), with stronger trends observed among smokers.

Conclusion: Significant associations between arsenic exposure and mortality have been observed in the HEALS cohort. While initiatives to reduce exposure to arsenic through drinking water are ongoing, investigation into solutions to mitigate the resulting health effects of arsenic exposure deserve urgent attention.

Cross-Species and Life Stage Physiologically Based Pharmacokinetic Modeling of Benzo[a]pyrene and Dibenzo[def,p]chrysene

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous and often carcinogenic environmental contaminants generated as byproducts of natural and anthropogenic combustion processes. PAHs are “re-emerging” environmental pollutants found at almost half of Superfund sites, and they are primary drivers for remediation in a significant percentage of those sites. Daily exposure to PAHs is increasing, as worldwide energy consumption (e.g., coal-burning power plants, petroleum based fuels) continues to rise. Benzo[a]pyrene (BaP) is the prototypical carcinogenic PAH, while dibenzo[def,p]chrysene (DBC) is a less prevalent but highly potent transplacental carcinogenic PAH. While metabolic activation to reactive intermediates is required for toxicity, little is known about the pharmacokinetics and target tissue dosimetry of PAHs and their metabolites in humans under real world exposure conditions. Physiologically based pharmacokinetic (PBPK) models are state-of-the-art tools for integrating ADME processes (absorption, distribution, metabolism, and elimination) with anatomy and physiology, thereby facilitating prediction of target tissue doses of toxic metabolites across species and exposure. Through a combination of *in vitro*, *in vivo*, and *in silico* studies in mice, rats, and humans, we have developed the first species- and life stage-specific PBPK models for any high molecular weight PAHs that can be used to predict target tissue doses of PAH metabolites important to their carcinogenic mode of action. In rodents, we have measured metabolic enzyme activity, rates of metabolism, and pharmacokinetics during vulnerable life stages such as pregnancy and fetal development. Pregnancy was found to have a significant impact on the pharmacokinetics of DBC in the mouse. Pregnant animals had elevated tissue concentrations of DBC and its metabolites and suppressed rates of clearance; these pharmacokinetic alterations led to increased residence time and elevated exposures to the developing fetus. We employed activity-based protein profiling (ABPP), a unique proteomic approach for characterizing functional proteins in complex biological systems, to measure and compare enzyme activity in naïve, pregnant, and developing mice, and found that multiple enzymes important for PAH metabolism are suppressed during pregnancy. Incorporating changes in anatomy and physiology during pregnancy as well as this 2-10 fold reduction in P450 enzyme activity determined by ABPP, we were able to address the significant differences in pharmacokinetic behavior. We have extrapolated our PBPK models to describe human dosimetry on the basis of human anatomy and physiology and chemical specific metabolic rates measured *in vitro* in rodent and human microsomes. In order to evaluate the extrapolated human PBPK model for DBC, the first controlled human pharmacokinetic study of any PAH at environmentally relevant exposure levels has been performed by our SRP, utilizing accelerator mass spectrometry (AMS) to

measure DBC and metabolite concentrations in urine and plasma of humans administered a 30 ng bolus of DBC. Initial simulations of DBC concentrations in blood are remarkably similar to those observed in preliminary data from the first human volunteer. This work constitutes a critical bridge between environmental levels of PAHs and the potential for adverse response in humans.

Mouse Models of Human *In Utero* Exposure to Low-Dose Arsenic in Drinking Water: Significant Effects on Lipid Metabolism, Lung Development, and Fetal Growth and Development.

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Arsenic exposure is a significant environmental health concern both in the U.S. and worldwide. Chronic exposure via contaminated drinking water has been associated with an increased incidence of a number of diseases, including lung and other cancers; non-cancer lung diseases, including bronchiectasis and COPD; growth defects, type 2 diabetes, cardiovascular disease, and other metabolic disorders; and reproductive and developmental effects. There is growing epidemiological evidence for arsenic playing a significant role in the fetal basis for adult disease – i.e., where *in utero* and early childhood exposures represent a uniquely sensitive window of exposure leading to greatly increased risk of increased morbidity and mortality for those individuals as adults. The goal of these studies was to develop unique mouse models of human fetal and early childhood exposure to arsenic in order to determine both the adverse consequences of these exposures in controlled laboratory experiments and to investigate the underlying mechanisms for these effects. In the first model, young adult female C57BL6/J mice were exposed to inorganic As+3 in drinking water at 10 ppb, which is the current US EPA Maximum Contaminant Level (MCL), during pregnancy (3 weeks), during the postnatal nursing period up to weaning (3 weeks), or both periods. Birth outcomes, growth of the offspring, and health of the dams were assessed by a variety of measurements. Birth outcomes, including litter weight, number of pups, and gestational length were unaffected. However, exposure during the *in utero* and postnatal period resulted in significant growth defects in the offspring after birth, which was principally a result of decreased nutrients in the dam's breast milk. Arsenic-exposed dams displayed significant hepatosteatosis as well as altered liver and breast milk triglyceride levels and serum profiles during pregnancy and lactation. The growth defects in the offspring resolved following separation from the dam and cessation of exposure in male mice but did not resolve in female mice up to six weeks of age. Cross-fostering of the pups largely reversed the growth defect. In parallel experiments, adult male C57 mice were also exposed to 10 or 100 ppb arsenic in drinking water and showed similar alterations in circulating triglycerides as the pregnant females and also demonstrated alterations in their white adipose tissue. In the second mouse model, human fetal lung tissue from spontaneous abortions (13-22 weeks gestation) was xeno-transplanted under the kidney capsules of immune-compromised SCID-beige mice, and the mice were then exposed to arsenic in drinking water at 10 or 100 ppb. In the control animals, the transplants remained highly differentiated, viable, and clearly identifiable as human fetal lung. In the arsenic-treated animals, we observed significant, dose-dependent alterations in lung development, cellularity, and mRNA expression associated with normal lung development and function, similar to changes associated with development of lung disease. Taken together, these results indicated that exposure to arsenic at the current U.S. MCL during critical windows of development induces a number of adverse effects in these mouse models, suggesting that such effects may occur and may contribute to increased disease risks in exposed human populations.

Unraveling Cadmium-Specific Epigenetic Changes Associated with *In Utero* Exposures

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Cadmium (Cd) is a known human carcinogen and developmental toxicant that can cross the placental barrier from mother to fetus. Cd exposure *in utero* has been associated with numerous health outcomes, including impaired physiological and neurobehavioral development in children; yet a pathophysiological mechanism remains unknown. This study examined the effects of maternal Cd exposure on DNA methylation patterns in newborn cord blood that were independent of cigarette smoke exposure. DNA was collected from the cord blood of seventeen newborns of the Healthy Pregnancy, Health Baby cohort in North Carolina. A methylated cytosine-guanine (CpG) island recovery assay was used to assess over 4.6 million sites spanning 14,362 CpG gene promoter islands. Newborn Cd levels were classified according to maternal blood levels, and comparative methylation analysis was performed to identify statistically significant differences in DNA methylation between Cd exposure groups. The data also controlled for cotinine exposure. Using a comparative strategy, we identified a subset of cadmium-only (n=27), cotinine-only (n=563), and shared (n=17) genes with altered DNA methylation levels. The majority of the identified genes were hypermethylated. Systems level analysis of the differentially methylated genes revealed that Cd exposure is associated with changes to genes involved in inflammatory response and bone development. This study provides evidence for epigenetic alterations in newborns via maternal exposure to Cd and highlights the potential for heritable effects of environmental Cd exposure during the prenatal period.

Session Two: Community Engagement

Engagement of Native American Tribes in the Determination of Legacy and Emerging PAH Dietary Exposure Scenarios, Assessment of Possible Risks to Human Health

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Although it is known that legacy toxicants, such as polycyclic aromatic hydrocarbons (PAHs), can be introduced into meats via smoke curing, little is known about their prevalence in smoked salmon prepared using traditional Native American smoking techniques. This work sought to characterize the effect of traditional Native American fish smoking methods on dietary exposure to 33 legacy and emerging PAHs and identify possible risks to human health. Salmon smoking events were carried out by Tribal researchers at the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) in collaboration with Oregon State University Superfund Research Program (OSU SRP) researchers. Fresh caught spring-run Chinook salmon were smoked using two commonly used smoking structures (tipi or shed) and two types of traditionally used woods (apple or alder). For the purposes of exposure and risk assessment, all salmon samples were prepared as if to be eaten. Additionally, 20 non-smoked spring-run Chinook salmon were analyzed for background PAH content along with three commercially available smoked salmon. Salmon samples were subsequently analyzed for PAH content using a novel analytical approach developed and validated specifically for application in this study.

Across all smoking methods, individual PAH loads ranged between < 2 – 3,800 µg/kg, where non-carcinogenic, carcinogenic, emerging, and legacy PAHs generally accounted for 95%, 5%, 20%, and 80% of the total PAH load respectively. It was found that neither smoking structure nor wood type accounted for differences in smoked salmon content of 33 PAHs. However, carcinogenic and non-carcinogenic PAH loads in traditionally smoked salmon were 40 – 430 times higher than PAH loads measured in fresh caught non-smoked salmon and commercial smoked salmon. Exposure to the levels of carcinogenic PAHs measured in traditionally prepared smoked salmon could result in excess lifetime cancer risks between 1E-5 and 1E-4 at a daily consumption rate of 5 g/d and could approach 1E-2 at 300 g/d. Exposure to non-carcinogenic PAHs could result in hazard indexes of 0.005 at 5 g/d and approach 0.3 at 300 g/d. Levels of PAHs present in smoked salmon prepared using traditional Native American methods potentially pose elevated cancer risks if consumed at high consumption rates over many years.

During the course of this study, the OSU SRP Community Engagement Core partnered with CTUIR to develop and publish a unique Material and Data sharing agreement. In accordance with the agreement, study findings were formally presented to CTUIR members and researchers on two separate occasions: once in Portland, Oregon at the Food Innovation Center and again in Pendleton, Oregon to the CTUIR advisory committee. Engagement of the CTUIR led to rich discussions, which ultimately resulted in the

development and submission of a joint publication for peer review with the American Chemical Society. Study results are currently being evaluated in order to design a culturally specific message for the Tribes.

United We Stand: Developing Regional Community Coalitions to Address Hazardous Waste Site Cleanup

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The University of Washington's Superfund Research Program (UW-SRP) Translation and Outreach Core (the Core) has been engaging communities living around contaminated waste sites in Washington, Oregon, Idaho, and Alaska (EPA Region 10) for over a decade. For the past seven years, we have assisted communities in building a regional coalition that addresses critical concerns that are common across waste sites. The Northwest Toxic Communities Coalition (NWTCC) was established as a nonprofit organization in 2008. The coalition began with a core set of members representing twelve community organizations from across the region. The NWTCC's mission includes establishing greater capacity and parity among communities impacted by toxic waste, promoting increased transparency and access to information, enhancing public participation at all levels of government, and educating and advocating on behalf of the public interest. Some of the founding members represent organizations that have been in existence for over twenty years. The Core has provided a forum to support annual NWTCC two-day summits, where members can meet face-to-face to discuss their priority issues and develop strategies to address them. The summits have working sessions that have included topics such as sediment treatment alternatives, improving timely access to cleanup plans, as well as specialized trainings. UW-SRP investigators, other scientists, and regional agency staff from ATSDR and EPA attend the annual meetings as presenters or as roundtable participants. Participation in the summits has grown over the years, helping to increase NWTCC membership to over 25 organizations. In 2010, the NWTCC realized one of its top goals when the new Region 10 EPA Administrator agreed to convene a series of meetings with coalition members to discuss ways in which the agency could work with the coalition to prioritize and better address environmental and health issues of concern. Core staff assisted in setting up these meetings and participated in the discussions with EPA staff and coalition members. Six meetings took place over a seven-month period. A need for robust and meaningful community involvement from the onset of site identification and insuring that states honor their responsibilities for cleanup were among the topics discussed. A list of priority issues was developed to guide follow-up activities. At the top of the list of priority issues was the common concern of citizens regarding air quality in their neighborhoods. EPA staff, UW-SRP, and NWTCC members worked together as planners to create a "first of its kind" *Northwest Regional Outdoor Air Quality Workshop for Communities.* The workshop was held at EPA Region 10 headquarters with video links to Oregon and Idaho. Staff from ATSDR and state and regional air authorities participated in the workshop. The Core provided support for community members to travel to the workshop, assisted with logistics, and participated in the workshop sessions. Core staff and NWTCC members participated in a debriefing immediately after the workshop, and the 2012 NWTCC Summit took place the following day to continue discussions among community members and to develop strategies to build upon what was accomplished at the workshop. This effective three-way collaboration among agency, community, and academia provides key lessons on the importance of a sustained effort when it comes to successfully engaging communities to address environmental and health issues of concern.

Sensemaking in the Shadow of a Superfund Site: Defining ATSDR Roles and Goals in an Agency-Saturated Community

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By working directly in Superfund communities, the Agency for Toxic Substances and Disease Registry is embedded within a complex tapestry of federal and state agencies, local government entities, and other organizations that community stakeholders encounter regularly. The diversity of statutory obligations and expertise among these organizations, particularly as they relate to stakeholders' health concerns, presents challenges for creating shared understanding between agencies and the communities they serve. Thus, addressing key elements of individual sensemaking during engagement activities is essential for those who work in communities.

Because sensemaking helps individuals determine the seriousness of a situation, decide how to react to the situation, and interpret the situation after it has changed, the process affects every level of environmental risk communication, from assessment through response through post-crisis learning and back through assessment again. Central concepts are the adoption, maintenance, and breakdown of role structures. Highly contextual in nature, the sensemaking process is rooted in the impact that past experience has on the conceptualization of the possible, creating an inherently retrospective exercise.

Individuals play out prescribed roles to maintain familiar structures. When situations threaten these roles, the structures can disintegrate, leading to a loss of order and direction (Weick, 1995). As role frameworks deteriorate, a cascading effect occurs in which meaning is lost, causing additional deterioration of role frameworks, which leads to further loss of meaning and generates serious repercussions for relational stability (Bryant and Miron, 2004). Relational deterioration can further decrease the stability of role frameworks already made fragile by past crisis, such as contamination that results in a Superfund National Priorities List (NPL) designation. As Blumer (1966) states, "In the flow of group life there are innumerable points at which the participants are redefining each other's acts. Such redefinition is very common in adversary relations, it is frequent in group discussion, and it is essentially intrinsic to dealing with problems" (p. 538).

Drawing from case study evidence generated around the Paducah Gaseous Diffusion Plant NPL site, this presentation will address the ways in which basic sensemaking processes—and particularly role definitions—play into public understandings of ATSDR specifically and, more broadly, "the government" – a term that is often used nonspecifically to encapsulate the activities of *all* agencies engaged in site activities. Data derived from interviews, focus groups, and media content analysis will be contextualized within risk communication and organizational sensemaking theory to illustrate specific relational and trust challenges that have arisen through misunderstandings of agency functions and subsequent interactions. The implications of such role blurring for practitioners in the field, as well as potential mechanisms for minimizing role confusion, will also be discussed.

University of Arizona Superfund Research Program: Engaging Arizona Communities near Contamination

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The University of Arizona Superfund Research Program (UA SRP) has used an interdisciplinary approach to study hazardous waste issues in the U.S. Southwest and Mexico border region for more than twenty-three years. We focus on arsenic, chlorinated solvents, and mine tailings. The goal of the Research Translation and Community Engagement Cores is to apply the knowledge gained from our research projects to increase human capacity and information dissemination to diverse stakeholders, including communities in Arizona, northern Mexico, and the border, as well as federal, state, and local government agencies.

For the community at large, we have developed a variety of bilingual informational materials – from pamphlets on environmental contaminants to booklets on water resources in Arizona to scientific bulletins on remediation strategies. We meet with the community face-to-face at local events, such as festivals and health fairs, where we distribute materials, answer questions, and act as liaisons to bring community concerns to our expert researchers. In addition, we provide requested informational presentations at public forums.

We have developed selected educational modules for use by *promotoras* and tribal community college educators in the area of toxicology, arsenic, pesticides, mining, and contaminant fate and transport. By working with these educators from the beginning and using audience feedback, these modules are developed to present information in a culturally relevant manner.

We work with a number of communities neighboring Superfund sites. These include sites impacted by chlorinated solvent groundwater contamination, such as the Tucson International Airport Area, Motorola 52nd Street, Phoenix-Goodyear Airport Area, and Indian Bend Wash Area, as well as sites impacted by legacy mining issues, such as the Iron King Mine and Humboldt Smelter. We build upon existing infrastructure, such as Community Action Groups/Community Involvement Groups, and coordinate with US Environmental Protection Agency personnel to provide neutral, scientifically accurate information. As they become familiar with the UA SRP, community members begin to solicit the information they need to understand the issues and become more empowered with knowledge.

We also collaborate with non-Superfund communities impacted by environmental contamination. Upon request, we provide the same types of materials and expertise as above, and in some cases, hold local meetings in response to specific concerns. Examples include the Valle Verde community in the border town of Nogales, where the local water company was in violation of TCE standards; the Sahuarita retirement community, which had concerns about particulates from local mine tailings; and the Tohono O'odham Nation, with concerns about the construction of a hazardous waste landfill near sacred sites, as well as abandoned wildcat mines.

We act as a neutral party to provide scientifically accurate information and have gained the respect of a number of Arizona communities impacted by environmental contamination, as well as local, state, and federal agencies. The successful partnerships that we have built have made us a resource and collaborator across the state. Our responses to community concerns have led to recognition of the contributions we are capable of making and, in turn, to additional requests for our support.

Session Three: Environmental Fate & Transport

Development of Elemental Mercury Sensor Based on Gold Nanoparticles

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Mercury is a potent neurotoxin of particular concern to pregnant women and developing children. Approximately 50% of the anthropogenic mercury that is released into the environment is from coal combustion for power, and due to long residence times in the atmosphere, it can be transported globally. Recent regulations had imposed emission control standards on stationary sources in the United States, increasing the interest in reliable, sensitive, and cost-effective methods to measure the concentration of mercury in flue gas or in the ambient environment. We are developing a low-cost, relatively simple sensor to detect elemental mercury based on gold nanoparticles. Gold and mercury readily form an amalgam, and the optical properties of gold nanoparticles create strong absorbance peaks in the visible range. These optical properties are very sensitive to environmental conditions, making them an ideal sensor for mercury. The size of these nanoparticles, less than 10nm in diameter, makes them suitable for being excited and analyzed via the evanescent wave of a fiber optic cable, which provides a convenient sensing platform. The system has a sensitivity down to 1µg/m3 in air, comparable to the commercial mercury constant emission monitors used on coal fired power plants. We also investigated the possible confounding effects of temperature and humidity using particles deposited on quartz microscope slides, analyzed with transmitted light. Humidity and temperature can affect the reading of the particles, though we show that this can be characterized and controlled. Quartz microscope slides also provided a platform for optimizing the mass transfer of mercury to the gold nanoparticles, decreasing the necessary exposure time at a given mercury concentration. Additional work has been done on the characterization of the response of individual gold nanoparticles to mercury exposure, elucidating some of the fundamental interactions, such as the limit of mercury mass transfer at saturation as a function of surface to volume ratio and the lack of a detectable shape change.

Monitoring Current-Use Pesticide Exposure Using Passive Sampling Devices

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Current-use pesticides have generally less persistent and more polar/hydrophilic chemicals than their chlorinated predecessors. Additionally, acute pesticide "poisoning" events are very rare, and pesticide application rates are very low, so the predominant environmental concern is long-term chronic exposure at very low concentrations of pesticides. This presents new challenges in estimating environmental exposure and assessing human and ecological risks associated with pesticides in aquatic systems. Several types of passive sampling devices (PSDs) have been developed for monitoring chronic chemical exposure in aquatic systems; however, their use is largely limited to hydrophobic chemicals (e.g., Huckins et al., 2002). There remains a need for a simple, robust method to measure chronic exposure to more polar compounds. A polar organic chemical integrative sampler (POCIS) was developed for hydrophilic organic contaminants (Alvarez et al., 2004; Mills et al. 2007); however the POCIS does not provide sufficient quantitative exposure estimates. We have developed a more universal passive sampling device (uPSD), which is composed of a porous stainless steel cartridge and a mixed-polymer sequestration sorbent. The uPSD has been calibrated in the laboratory for 52 current-use pesticides with log Kow values ranging from 0.28-8.15. Performance reference compounds have been selected and

demonstrated to adequately correct for environmental conditions that influence uptake rates. We have deployed these samplers at numerous field sites, and chronic exposure estimates using the uPSDs closely match those using sequential grab sampling, with some exceptions. We will report on these field verification studies and also the current limitations of the uPSD and possible expansion in applications.

Impacts of CVOCs and Phthalates Contamination in the Karst Groundwater System of Northern Puerto Rico

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Puerto Rico has one of the highest rates of preterm birth in United States. Preliminary investigations suggest that the increase in preterm birth rates in Puerto Rico cannot be explained by changes in known factors and that there is sufficient evidence that exposure to some contaminants contributes to preterm birth. Contamination in Puerto Rico is extensive with more than 150 contaminated sites and a vast contamination of water resources. Of particular concern is the contamination in the north-coast karst aquifer. This system is the most productive aquifer of the island and serves as a significant source of water for human consumption. The same characteristics that make this aquifer highly productive also make it vulnerable to contamination. This research addresses the historical contamination of groundwater resources and its impact on the drinking water system in the northern karst region of Puerto Rico. The study focused on chlorinated volatile organic compounds (CVOCs) and phthalates because of their ubiquitous presence in the environment, presence in listed and potential Superfund sites in Puerto Rico and the U.S., and potential for exposure and health impacts. Results show an extensive historical contamination of the groundwater resources in the northern karst aquifers that has reached drinking water sources. Long-term contamination indicates a large capacity of the aquifers to store and release contaminants and reflects a long-term potential for exposure and associated health impacts. The degradation of this important water resource reduces the extraction capacity for drinking water, increases the cost of use, and impacts the ecologic integrity of the system.

Remediation Effectiveness for Mining Sites: Hysteresis and Metal Mixture Effects

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In almost all ecosystems, metals occur in mixtures; this is especially true in mining impacted watersheds. Accurate assessment of metal exposure and effects to humans and ecosystems must account for mixture effects, which could be additive, less than additive, or more than additive. However, current regulatory and management practices usually address individual metals because adequate tools are not yet available for predicting bioavailability and toxicity of mixtures. The project attempts to address this shortcoming by improving the ability to measure, understand, and predict bioavailability and toxicity of metal mixtures arising from environmental contamination.

The study site is the North Fork of Clear Creek, located within the Central City/Clear Creek Colorado Superfund site. We are developing and testing the “gellyfish” sampler, which is a resin-based passive sampler that adsorbs the free metal ion fraction. Experiments are being performed both in the laboratory

and in field deployments. Secondly, we are further developing a model of the toxicity of metal mixtures to individual species of aquatic organisms based on a mixed-metal, multi-site biotic ligand model (MMMS BLM). This is being accomplished with laboratory *D. magna* toxicity tests using both simple solutions and water samples collected from the site. Finally, we are examining the aquatic communities currently present in the stream and relating these observations to the chemical data obtained from both the "gellyfish" and the laboratory toxicity testing.

Within the next 1-2 years, a treatment facility will be completed to reduce the metal loadings from two inputs of metal-rich waters. This will allow us to perform a "natural experiment" in a metal-contaminated stream and examine responses before, during, and after treatment. One important aspect of the system is the abundance of iron oxides in the sediments formed by ferrous iron oxidation and ferric hydroxide precipitation. Because some metals like Fe can be toxic to aquatic invertebrates and fish in streams but can also make other metals less bioavailable (i.e., be protective), we expect to see a hysteresis in the recovery of the stream as Fe loading is decreased by the treatment system. Ultimately, in this project, we expect to make improvements in assessment methods, which will provide a better means for protecting human and ecosystem health.

Aqueous Solubility of Binary and Multicomponent, Tarlike PAH Mixtures

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Polycyclic aromatic hydrocarbons (PAHs) are common components of many materials, such as petroleum and various types of tars. They are generally present as mixtures, occurring both naturally and as byproducts of fuel processing operations. It is well known that manufactured gas production resulted in subsurface contamination by PAH-containing tars and non-aqueous phase liquids (NAPLs). In an effort to better characterize the phase and partitioning behavior of PAH mixtures, one must consider that PAH mixtures sometimes exist in places where contact with water is to be expected (e.g., the subsurface) and that this might affect the fate and transport of these compounds and the stability of the source material. Hence, the ability to understand PAH mixture dissolution is important for many environmental and commercial applications.

Since tars and NAPLs may evolve to a limited number of components, this experimental study is aimed at understanding the aqueous phase solubility of transitional multicomponent mixtures, not containing the wide range of compounds that characterize authentic tars. We have been interested in determining whether these mixtures partition into water as individual, phase-separated species or following some solution behavior. Solution theory is quite different for liquids than for solids, and this raises the question as to which predictive model might be best.

The measured aqueous solubilities of binary and multicomponent PAH mixtures have shown fair, unexplained variability in this study, falling both above and below predicted values. Nevertheless, recent results show that binary and ternary PAH mixtures trend towards phase-separated dissolution behavior – that is, the components of the mixtures dissolve independently of one another and approach predicted pure species solubility concentrations in the aqueous phase. As more PAHs are added to create tarlike mixtures, the aqueous solubilities of these higher-component mixtures seem to better fit the values predicted by solid-solution theory, though there is still significant failure to predict actual values. These results serve as a preliminary indication of behavior, suggesting that as PAH mixtures become enriched in components to a point at which only a discrete number of compounds exist in the mixture, solubility might be roughly predicted by ideal solid-solution theory or by pure component values. Though there was no reason to initially hypothesize that the multicomponent, tarlike mixtures in this study would follow the

liquid-solution theory commonly reported for NAPLs, the behavior observed here is quite different from what one would expect if that model were used. Of course, this is significant for those dealing with PAH mixtures in natural settings.

Session Four: Emerging Contaminants

Radical-Containing $PM_{0.2}$ Initiates Epithelial-to-Mesenchymal Transitions in Infant Airway Epithelial Cells

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Rationale: Combustion generated particulate matter (PM) from industrial processes and burning of biomass and fossil fuels have been linked with adverse pulmonary health effects. We have recently identified a previously unrecognized component of airborne PM formed in combustion and thermal processes, namely, environmentally persistent free radicals (EPFRs). *In vivo* exposure of infant rats to EPFRs demonstrates their ability to induce airway hyperresponsiveness to methacholine – a hallmark of asthma. However, the mechanism(s) by which combustion-derived EPFRs elicit *in vivo* responses remains elusive.

Methods: In this study, we employ a chemically-defined EPFR consisting of ~0.2µm amorphrous silica containing 3% cupric oxide with the organic pollutant, 1,2-dichlorobenzene (DCB-230). DCB-230 possesses similar radical content to urban-collected EPFRs but offers several advantages, including lack of contaminants and chemical uniformity.

Results: DCB-230 was readily taken up by BEAS-2B, and at high doses (200µg/cm^2) caused substantial necrosis. At low doses (20µg/cm^2), DCB-230 particles caused lysosomal membrane permeabilization, oxidative stress, and lipid peroxidation within 24h of exposure. During this period, BEAS-2B underwent epithelial-to-mesenchymal transitions (EMTs), including loss of epithelial cell morphology, decreased E-cadherin expression, and increased α-smooth muscle actin (α-SMA) and collagen I production. Similar results were observed in neonatal air:liquid interface culture (i.e., disruption of epithelial integrity and EMT). Importantly, acute exposure of infant mice to DCB-230 resulted in EMT as confirmed by lineage tracing studies and evidenced by co-expression of epithelial E-cadherin and mesenchymal α-SMA proteins in airway cells and increased SNAI1 expression in the lungs.

Conclusions: EMTs in neonatal mouse lungs following EPFR exposure may provide an explanation for epidemiological evidence supporting PM exposure and increased risk of asthma. Further studies are needed to address the downstream reversibility of these changes upon later exposure to PM, antigen, or pathogens.

The Potential Role, Mechanisms, and Relevance of Pollutant-Induced Oxidative Stress in Preterm Birth

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Preterm birth, which occurs in 1 in 8 pregnancies in the United States, is costly, often disabling, and may predispose a premature infant to adult onset diseases. The potential for environmental chemical exposures to contribute to preterm birth remains vastly understudied, even though known risk factors fail to explain about 40% of preterm births. Our research team is part of a collaborative investigation of

preterm birth in the Superfund Research Program center, Puerto Rico Testsite for Exploring Contamination Threats (PROTECT). Puerto Rico is an island disproportionately impacted by hazardous waste sites and a preterm birth rate of ~20%. We are investigating phthalates, environmental chemicals with widespread exposure to humans, for their ability to stimulate oxidative stress, target tissues of the gestational compartment, and increase risk for preterm labor in humans. The active phthalate metabolite mono-(2-ethylhexyl)-phthalate (MEHP) significantly stimulated dichlorofluorescein (DCF) fluorescence in human placental trophoblasts (HTR-8/SVneo cell line) at concentrations ranging from 45-180 µM MEHP ($p<0.05$), suggesting generation of reactive oxygen species that could contribute to oxidative stress. Furthermore, MEHP exposure increased oxidation of the DNA base thymine and increased expression of genes in the antioxidant response pathway (PTGS2, GLRX2, and TXNRD1) in HTR-8/SVneo cells ($p<0.05$), consistent with oxidative stress. The increased mRNA expression of PTGS2 (also known as COX-2) with 90 µM and 180 µM MEHP ($p<0.05$) is particularly noteworthy because of the key role of PTGS2 in the production of prostaglandins that drive parturition. Consistent with the PTGS2 mRNA induction, MEHP stimulated release of prostaglandins from HTR-8/SVneo cells, as well as from human placental macrophages and extraplacental membrane explants in culture. A role for oxidative stress in parturition is further supported by findings with the model pro-oxidant *tert*-butylhydroperoxide (TBHP), which readily crosses cell membranes and spontaneously generates reactive oxygen species within the cell. TBHP significantly stimulated release from HTR-8/SVneo cells of prostaglandins and the pro-inflammatory cytokine interleukin-6 (IL-6) ($p<0.05$), both of which are associated with parturition onset in women. The TBHP-stimulated release of prostaglandin E2 but not IL-6 was blocked by inhibition of MAPK p38 but not MEK1/2 or JNK activity, suggesting that reactive oxygen species may increase PTGS2 gene expression by a p38 MAPK phosphorylation pathway. The translational potential of these findings for human population studies of preterm birth is supported by our recent reports of associations between biomarkers of oxidative stress and urinary phthalate metabolite concentrations in the NHANES biomarker database. Our PROTECT center collaborations allow transdisciplinary interrogation of unresolved questions about the potential for environmental contaminants to contribute to risk for preterm birth, enabling, for example, our laboratory research team to explore biological explanations for epidemiologic associations while providing potential biomarker information for future epidemiologic study.

Children's Exposure to Flame Retardant Chemicals (Old and New) in Indoor Environments

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Polybrominated diphenyl ethers (PBDEs) are a class of flame retardant chemicals used to reduce the flammability of consumer products. Due to their persistence, bioaccumulation potential, and potential toxicity, PBDEs have been—or are currently slated for—phase-out in different regions of the world. Exposure to PBDEs is now known to be associated with changes in thyroid hormone levels and adverse neurodevelopmental outcomes, both in animal and human studies. While a large research effort has focused on adult exposure to PBDEs, less is known about children's exposure to flame retardants, particularly for chemicals used as replacements for PBDEs. To address this, our research group conducted a study to measure children's exposure to flame retardant chemicals in indoor environments. Our specific objectives were:

1) To determine whether children's (ages 1-3) serum PBDE levels were significantly associated with house dust and/or PBDE residues measured on hand wipes collected from the children;
2) To determine whether serum PBDEs were associated with gender, age, breast milk ingestion, race, and education level of the parents; and
3) To investigate children's exposure to new use flame retardants found in indoor dust.

To accomplish this, we collected paired blood samples, hand wipes, and house dust from children between the ages of 12-36 months who resided in the central North Carolina area between 2009 and 2010. A short questionnaire was administered to collect demographic and behavioral information. PBDEs were detected in every serum sample, and the most abundant congeners were those associated with the PentaBDE mixture. Concentrations ranged from 5.2 to 745 ng/g lipid with a geometric mean value of 43.3 ng/g lipid. Significant associations were observed between PBDE levels and both race and parents education level, with higher levels observed in non-whites relative to whites, and lower PBDE levels observed with higher paternal education levels. Serum ∑PentaBDEs were significantly correlated with ∑PentaBDE in both handwipe measurements and house dust but were more strongly associated with handwipe levels. Multivariate models revealed that handwipe levels, child's sex, child's age, and father's education accounted for 39% of the variation in serum $\sum BDE_3$ levels (sum of BDE47, 99 and 100). In contrast, age, handwipe levels, and breast feeding duration explained 39% of the variation in serum BDE 153. We also investigated new use flame retardant levels in house dust samples from this study to estimate children's exposure to PBDE replacements. Our analyses focused specifically on flame retardant chemicals that we had previously identified in new furniture and baby products. Our laboratory has analyzed more than 100 samples collected from both general furniture items and baby products to identify the most common flame retardants used in these products. Our research indicates that the two major flame retardants used in furniture and baby products are tris (1,3-dichloroisopropyl) phosphate (TDCPP) and a brominated flame retardant mixture known as Firemaster® 550 (FM 550). Levels of these current-use flame retardants in the indoor dust samples are comparable to the levels of PBDEs, and suggest that children's exposure to these new flame retardants is similar to PBDEs.

Triclosan Impairs Excitation-Contraction Coupling and Ca^{2+} Homeostasis in Cardiac and Skeletal Muscle

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Triclosan (TCS), a high-production-volume chemical used as a bactericide in personal care products, is a priority pollutant of growing concern to human and environmental health. TCS is capable of altering the activity of type 1 ryanodine receptor (RyR1), but its potential to influence physiological excitation-contraction coupling (ECC) and muscle function has not been investigated. Here we report that TCS impairs ECC of both cardiac and skeletal muscle *in vitro* and *in vivo*. TCS acutely depresses hemodynamics and grip strength in mice at doses $\geq$12.5 mg/kg ip, and $\geq$0.25 µM in water compromises swimming performance in larval fathead minnows. In isolated ventricular cardiomyocytes, skeletal myotubes, and adult FDB fibers loaded with the Ca^{2+} indicator Fluo-4, TCS depresses electrically evoked ECC within 10 min. Nanomolar to low micromolar TCS initially potentiates electrically evoked Ca^{2+} transients followed by complete failure of ECC, independent of Ca^{2+} store depletion or block of RyR1 channels. TCS also completely blocks excitation-coupled Ca^{2+} entry. Voltage clamp experiments showed that TCS partially inhibits L-type Ca^{2+} currents of cardiac and skeletal muscle, and [^{3}H]PN200 binding to skeletal membranes is noncompetitively inhibited by TCS. TCS potently impairs orthograde and retrograde signaling between L-type Ca^{2+} and RyR channels, revealing a new mechanism by which TCS weakens cardiac and skeletal muscle contractility in a manner that may negatively impact muscle health, especially in susceptible populations.

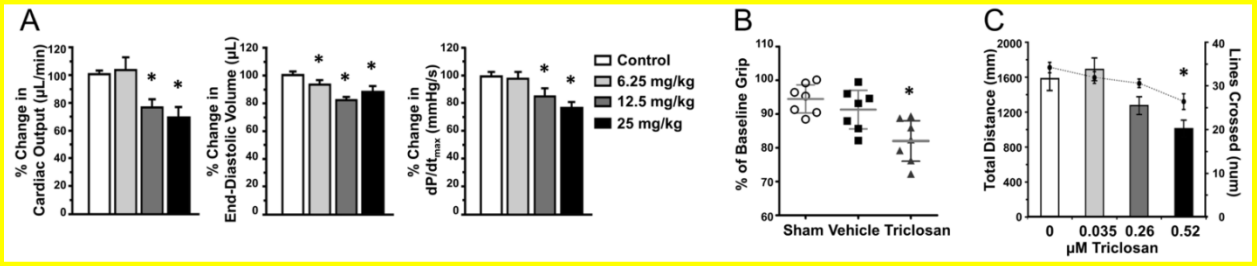

Figure 1. TCS depresses hemodynamics and compromises *in vivo* skeletal muscle function. (A) Mice were anesthetized with ketamine/xylazine and instrumented with recording catheter advanced into the left ventricle via carotid artery. After dosing with TCS (20% DMSO v/v in 200 µL) by i.p., both 12.5 and 25 mg/kg groups experienced significant reductions in cardiac output, ventricular filling, and blood pressure (* $p < 0.05$). **(B)** Grip strength was assessed in mice before and after sham, vehicle (30 µL DMSO), or 40 mg/kg TCS i.p. injection. Post-dose grip was significantly lower in the TCS group compared with both sham and vehicle (* $p < 0.01$, ANOVA). **(C)** Larval fathead minnow were exposed up to 7 d to vehicle (0.01% MeOH), or 0.035, 0.26, or 0.52 µM TCS; swimming behavior was assessed by non-provoked (distance traveled) and forced (lines crossed in 60 s) activity. A decreasing trend was seen for both swimming parameters, with significance found in the 0.52 µM group (* $p < 0.01$, Kruskal-Wallis).